LABORATORY APPARATUS IN VEGETABLE PHYSIOLOGY.

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(WITH PLATES XXIV AND XXV)

In the development of the laboratory methods in any department of science a need arises for convenient pieces of apparatus of special construction, and also of new adaptations of apparatus already on the market. It takes some time for the need and the supply to become adjusted, and suggestions and information in the earlier stages or days of development are particularly helpful. It is with a desire to contribute to this demand that the following descriptions of apparatus for work in vegetable physiology are given.[1] The several pieces have been devised in the laboratory of Purdue University to supply the requirements of the classes in physiology.

Auxanometer.—The main features of this apparatus were worked out by Miss Katharine E. Golden, while assistant in the laboratory in 1889–93, aided somewhat in the preliminary construction by her brother, Professor Michael J. Golden, of the Mechanical Department of Purdue University. Minor changes in perfecting it have been made as they occurred to those who used it, and especially by Mr. C. W. Meggenhofen, an unusually ingenious and painstaking mechanic, who for two years gave his best efforts to the construction of apparatus for this laboratory, and is still giving some time to it. He is to be credited also with much of the successful detail in other apparatus to be described, especially of the centrifuge.

[1] Some of the apparatus here described in detail was exhibited to the Botanical Club of the A. A. A. S. in 1893 and 1894. It has been in part briefly described also in the Proceedings of Indiana Academy of Science for 1894, not yet generally distributed, and in a descriptive circular and price-list of physiological apparatus, dated January 1, 1895.

The object has been to secure a piece of apparatus that will be simple, easily adjusted, giving so small an amount of tension to the tender part of the plant being studied as to be inappreciable, record with accuracy, and withal comparatively inexpensive.

The apparatus (*pl. XXIV*) stands about 45^{cm} high, and consists of two separate parts. The recording part supports a clock (either a twenty-four hour or eight-day clock) above, with its dial uppermost. To the lower end of the shaft which carries the minute hand is attached a frame supporting two glass rods, each 30^{cm} long. These rods are blackened by holding them over burning camphor gum, and receive transverse marks from a descending needle as they revolve once an hour.

Blackened glass rods in place of a revolving drum were first used on this apparatus, so far as the writer knows. They are very easily handled, and permanent records are readily secured by making blue prints, or the rods themselves can be kept. The principal value of two rods, rather than one, is to act as a control or duplication of the record, although separated by a half-hour interval.

The other part of the apparatus carries the multiplying pulley and needle. The pulley is made of aluminum for sake of lightness, and runs on carefully adjusted parallel bearings. There are one large and two small wheels upon the pulley shaft, permitting a magnification of the amount of growth either eight or fifteen times.

In the earlier machines, shown at the Madison meeting of the American Association for the Advancement of Science in 1893,[2] and illustrated in plate XXIV, the recording needle was a bristle adjusted in an aluminum holder, which slid upon a vertical glass rod, a second glass rod acting as a guide. This was found to be too heavy and clumsy, and has been replaced by a needle of fine brass wire attached to a skeleton carriage of similar wire, running upon two parallel tight wires in place of the glass rods. The needle and carriage together are barely heavy enough to straighten the slender thread used to suspend them, and it is

[2] See BOT. GAZ. 18: 348. 1893.

usually necessary to put weights of a few milligrams upon the carriage in order to make the machine run uniformly.

Many trials have been made to secure a satisfactory thread for the pulleys. The material found to work best is sewing silk A sufficient length is taken and untwisted, and only a part of one strand used. All attempts to secure a smoother thread by employing wax of some sort have met with indifferent success, and finally have been abandoned. The thread which leads from the plant to the small pulley is carried through a bent glass tube which offers but the slightest possible friction, and obviates the necessity of placing the plant directly underneath.

To adjust the apparatus the thread from the needle carriage is threaded through an opening in the rim of the large pulley wheel and fastened in a slit, the carriage remaining at the bottom of the guides. A thread from the plant is then passed through the glass rod and once around one of the smaller pulley wheels, and after the pulley is turned so that the needle carriage is at the top of the guides, the thread is fastened in a slit at the side of the small wheel. The carriage is now weighted enough to keep the threads barely straight. The recording part is next brought into place, and set so that the point of the needle will make a mark as the blackened rods pass it. To have the recording rods parallel to the path of the needle the apparatus should be set upon a level surface, preferably on a piece of plate glass. As the plant grows the needle descends at a multiplied rate, and leaves the record on the blackened rods.

There are no hanging weights, and no unnecessary pull upon the plant in this machine. The needle carriage and its riders constitute the sole weight for turning the pulley. As the plant grows the thread which leads from it is wound up on the small pulley wheel, and the thread attachèd to the needle carriage is unwound from the large wheel, permitting the needle to descend.

The small excess of weight permissible above that necessary to overcome the friction of the machine, in order not to influence perceptibly the growth of the organ under observation, makes it imperative that the movable parts of the machine should be kept in perfect order. It is especially necessary that the pulley bearings be clean and carefully adjusted, and that the guide wires for the needle carriage be kept polished. As the machine is intended to record increments of growth too small to be seen by the unaided eye, it is obvious, moreover, that it should be kept free from tremor or jar. It must, in fact, be treated as a machine of delicate precision in order to secure results of the highest value.

Centrifuge.—This machine (*pl. XXV*) is for the purpose of applying to small plants a force, in connection with or independent of gravitation, that shall induce a strain within the organ of the same physiological nature as that brought about by gravity. A disk of cork 10^{cm} in diameter, to which germinating seeds may be pinned is made to revolve at considerable speed in a moist chamber. The moist chamber consists of a shallow metal pan, into which a deep glass cover is fitted. The bottom of the pan is pierced by two openings, one of which conducts a constant supply of water into the chamber through a rubber tube terminating in a bent glass tube within. The glass tube can be adjusted from without so as to permit the water to fall in drops upon any part of the disk. An opening at the opposite side of the pan takes away the surplus water through a rubber tube.

The revolving disk is turned by a small electric motor, supplied with current from two Edison-Lalande cells of type Q. These batteries are the most satisfactory for small motors yet devised. When setting them up it is best to put the jar into a vessel of water, and let it remain there until the potash has fully dissolved and the excessive temperature abated, to avoid breaking the jar. After the cell is in readiness it requires no further attention until exhausted, when the potash, oil, zincs and carbon must be replaced with new ones. Although this is by far the best motor battery known, yet, like all batteries, it is likely to prove treacherous at times. Where suitable water power, or other more reliable motive power is available, it should be adopted.

When the cork disk is whirled horizontally the centrifugal force acts in a plane at right angles to that of gravity, and the direction of growth is a resultant of the two forces ; when the disk is whirled vertically gravitation is neutralized. In order to secure these positions the first machines made, which were exhibited[3] at the same time as the auxanometer already mentioned, had the moist chambers supported on a metal arc, and held in place by a set screw in the base (*pl. XXV*). But it was found difficult to accurately center the pulley wheel for the different positions, and later machines have been made with the chamber supported on a pinion (*fig. 1*).

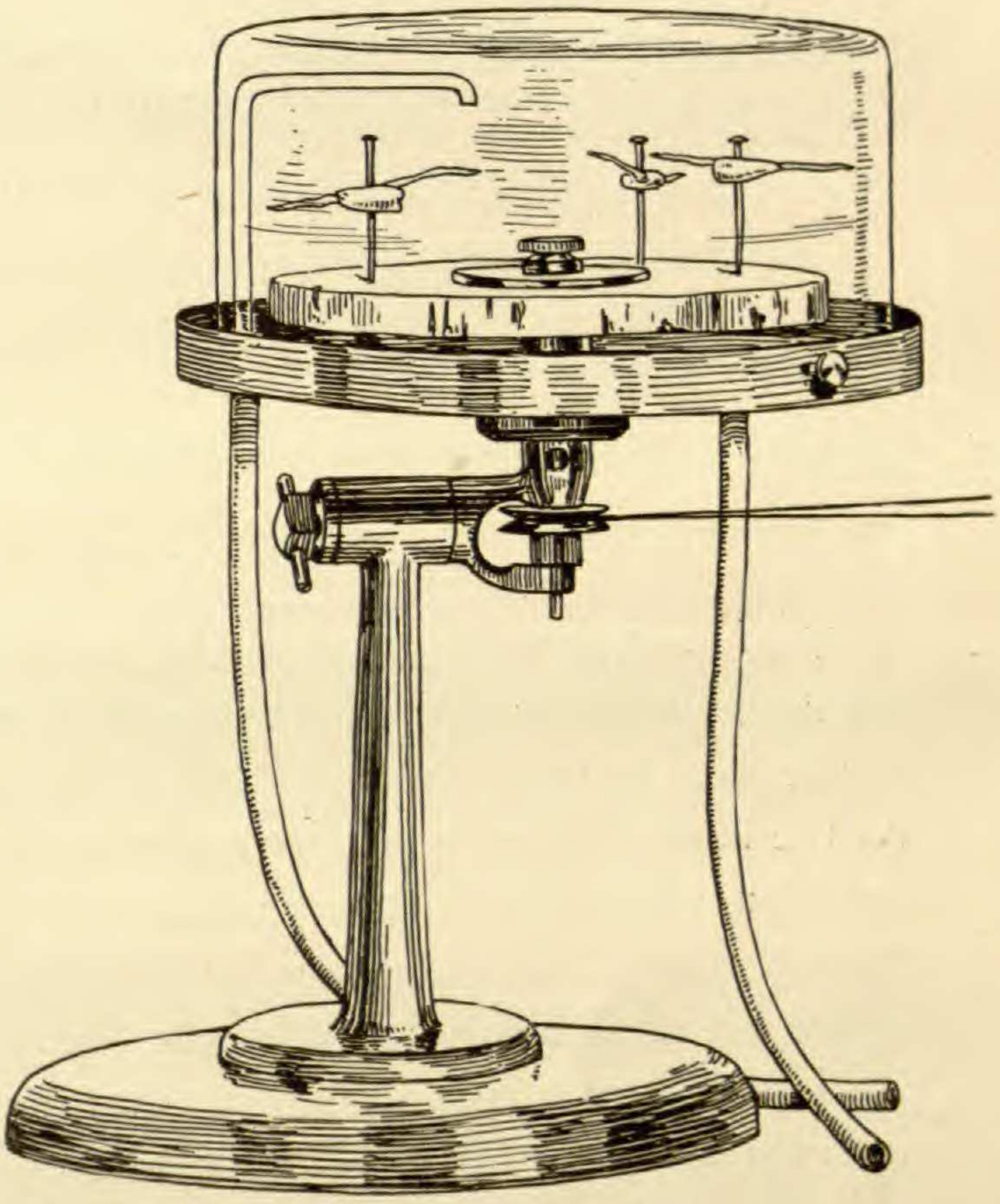

FIG. 1. Centrifuge with iron base.

When the electric motor is used it is mounted, along with the chamber, on a wooden base (*pl. XXV*); when other motive power is employed the chamber is mounted on a heavy iron base (*fig. 1*). To ascertain the speed of the disk a piece of paper is passed over a revolving pencil point for an exact number of seconds. The pencil point is attached eccentrically to the lower end of the spindle carrying the cork disk (*pl. XXV*) and describes a spiral as the paper is moved over it. By counting the number of turns of the spiral, the number of revolutions of the disk for the time is found.

[3] *Ibid.* 344.

Respirometer.—This piece of apparatus (*fig. 2*) is a modification of one employed by Pfeffer,[4] which in turn was adapted from Pettenkofer's apparatus for experiments upon the respiration of animals. It consists of a small chamber in which germinating seeds or other living material is placed, surrounded by water to keep the temperature uniform. A thermometer is plunged in the chamber, and one in the water outside.

FIG. 2. Respirometer.

Two U-tubes containing potash, and a wash bottle containing barium hydrate solution, are connected in series with the chamber at one end and with an aspirator, or water pump, at the other. At the beginning of an experiment the aspirator draws the air out from the chamber through a direct connection, and forces it through the potash tubes and wash bottle back into the chamber, freeing it of carbon dioxide in the operation. When the chamber is sufficiently free of carbon dioxide, the direct connection with the aspirator is broken, and a stopcock opened which permits the carbon dioxide free air, forced into it by the aspirator, to pass into and traverse a long tube (about a meter) partly filled with a solution of barium hydrate (made by adding 15^{gr} of barium chloride to 5^{l} of distilled water, and after it has largely dissolved adding 105^{gr} of barium hydrate), the gas then passing through a short supplementary tube, also containing barium solution. The bulbs at the distal ends of these tubes are to prevent an overflow, if a sudden increase in pressure should occur. There are two sets of tubes so that continuous observations may be carried on as long as desired. When the test has been in progress for the first period (usually an hour), a three-way cock

[4] Unters. a. d. bot. Inst. zu Tübingen 1: 637.

is turned, directing the stream of gas into the other set of tubes. During this period the first set of tubes is emptied and refilled and made ready for the third period, and so on. The object in having the barium solution in long tubes is to bring the gas well in contact with the solution, so that any carbon dioxide given off by the living material under observation will be taken up and precipitated as barium carbonate. The supplementary tube is to catch any carbon dioxide that may escape precipitation in the the longer tube, usually a very small amount, if any. A definite amount of solution is used in each tube, generally 100^{cc} in the long tube and 20^{cc} in the short one, or enough to fill the straight portions of the tubes. The two are emptied together at the close of the period into a closed vessel, and allowed to stand until the precipitate has fallen to the bottom.

To determine the amount of carbon dioxide that has been absorbed by the barium solution during one period, an integral part of the solution (20^{cc} is a convenient amount), after standing until clear, is titrated. A few drops of rosolic acid are added to the solution to be tested, as an indicator, and a standard acid solution (1.4318^{gm} of dry oxalic acid in 500^{cc} distilled water) is run into it from a graduated burette until exactly neutralized, as shown by the change of color. The same amount of the original barium solution is similarly titrated. The difference in the number of cubic centimeters of the standard solution used for each represents the number of milligrams of carbon dioxide absorbed by the integral part of the barium solution titrated, for one cubic centimeter of the standard acid solution will convert as much barium into an oxalate as one milligram of carbon dioxide will change to a carbonate. To find the total number of milligrams of carbon dioxide given off during the period, multiply the difference just obtained by the factor that represents the ratio of the full amount of barium solution employed in the trial to the amount titrated (six in the case supposed).

This apparatus is found to work with a steady water pressure, but sudden changes in pressure are very detrimental. Its accuracy is also all that is usually required.

The temperature of the respiration chamber can readily be changed by cooling or heating the water surrounding it.

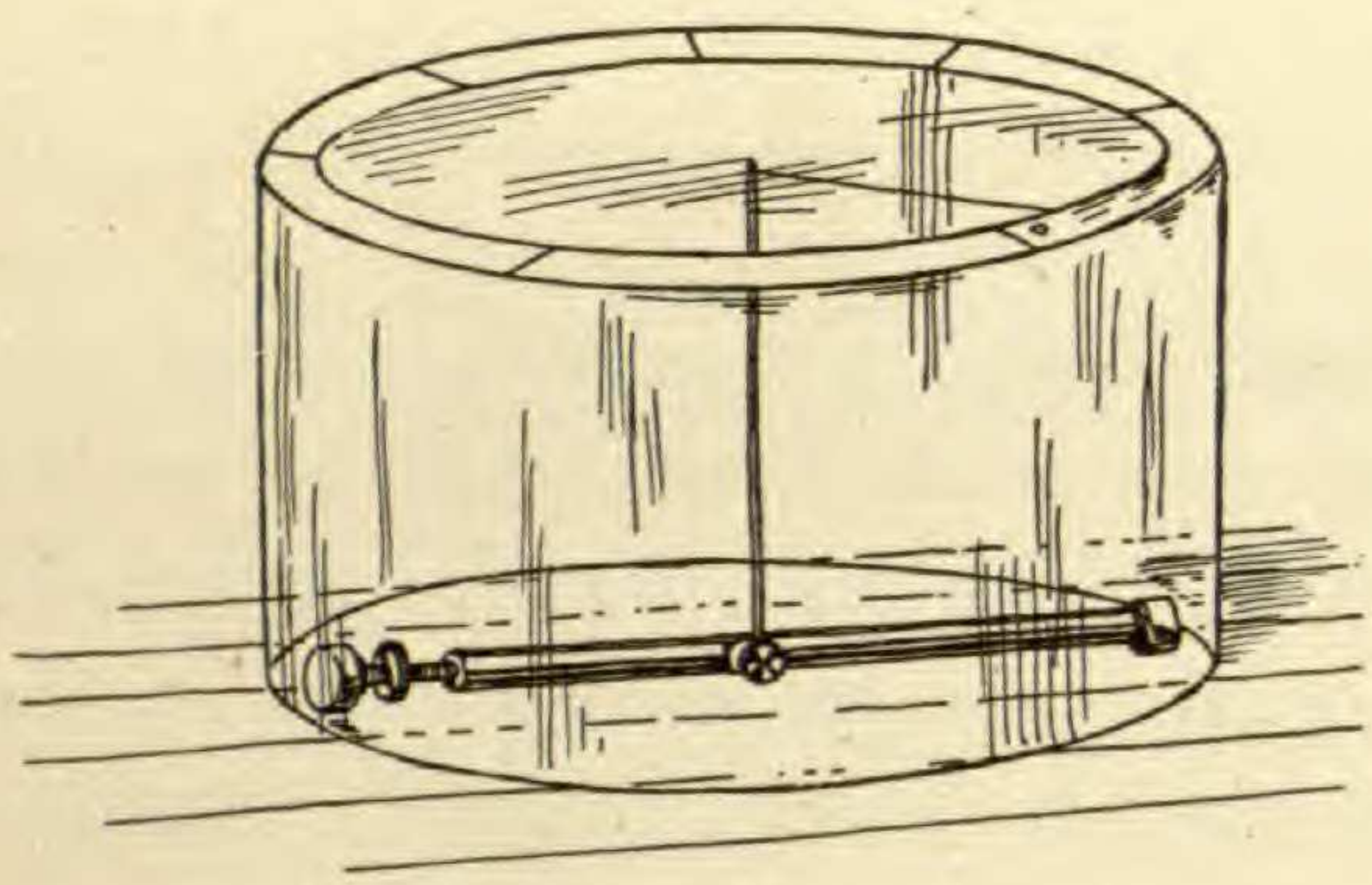

FIG. 3. Awn hygrometer.

Hygrometer. — When the writer was in the laboratory of Francis Darwin at Cambridge in 1888, he was shown a roughly made hygrometer,[5] which has since been used in the Purdue laboratory and modified into the form shown in *fig. 3*. It consists of a small thin glass vessel with a metal bar across the mouth bearing a part of a Stipa (*S. spartea* Trin.) awn. To the free end of the awn is attached an index of fine brass wire. When the air becomes moist, the awn untwists and the index is carried around. To use it two are selected with awns that untwist at the same rate, and fastened to opposite sides of a leaf by means of a mixture composed of wax, oil, and tallow. The leaf may be attached to the plant, or separated from it and kept from wilting by placing the end in water (*fig. 4*). The position of the index on each side of the leaf is marked at the start, and again when one of them has made a complete revolution. The ratio between the number of degrees of the circle traversed by the two indices during the interval is approximately the ratio of transpiration from the two sides of the leaf.

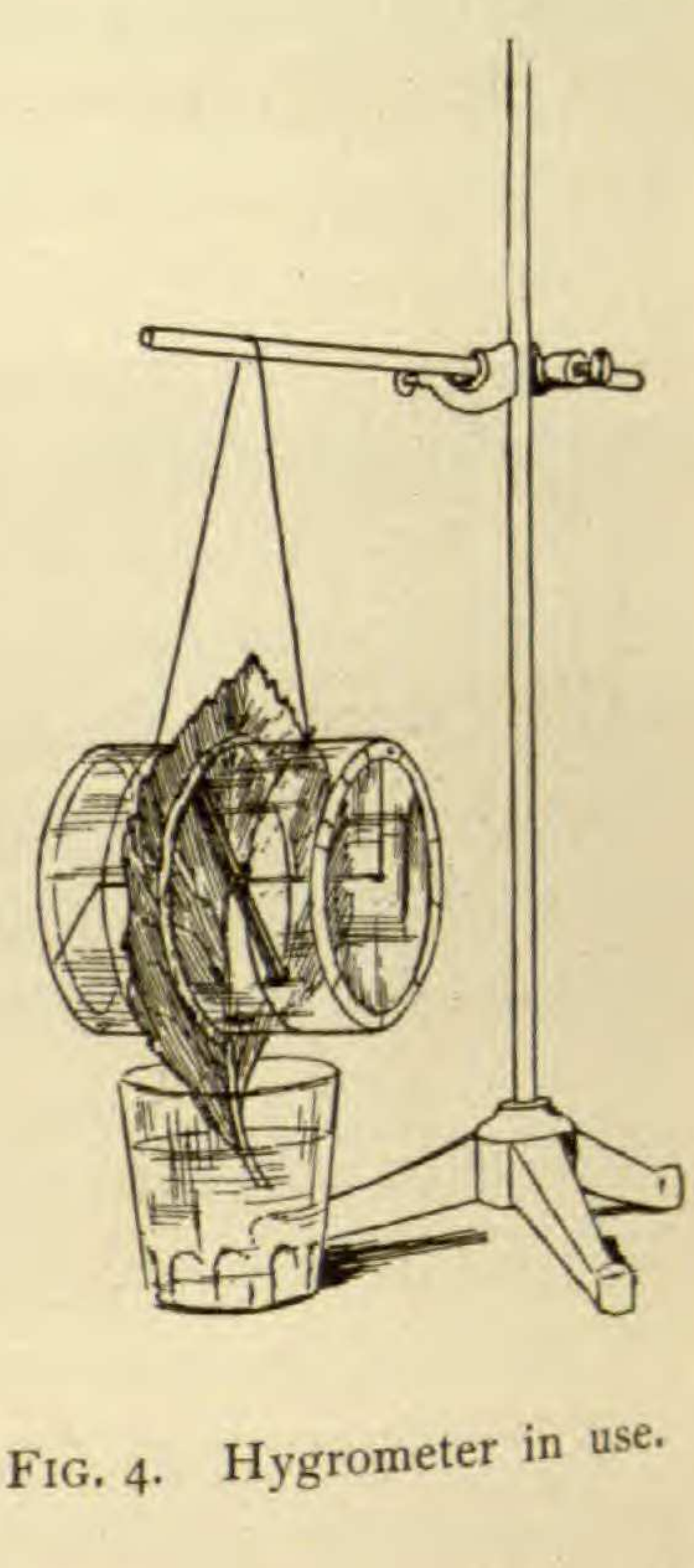

FIG. 4. Hygrometer in use.

[5] Since described and its use fully explained; see Darwin and Acton's Practical Physiology of Plants

The apparatus is not accurate, but makes an interesting demonstration of the general difference in transpiration between the two sides of different kind of leaves.

Slide with binding posts.—The difficulty in attaching wires to tin foil, when wishing to use an electric current under the microscope, has led to the device shown in *fig. 5*. An ordinary microscope slide is provided with a pair of small brass binding posts, each bearing a clip. When in use two wedge shaped pieces of tin foil are placed under the clips with their points near together. The object to be examined is mounted in a drop of water between the points, and covered with a cover slip in the usual manner. It is then placed on the stage of the microcope and the wires from the battery passed into the binding posts.

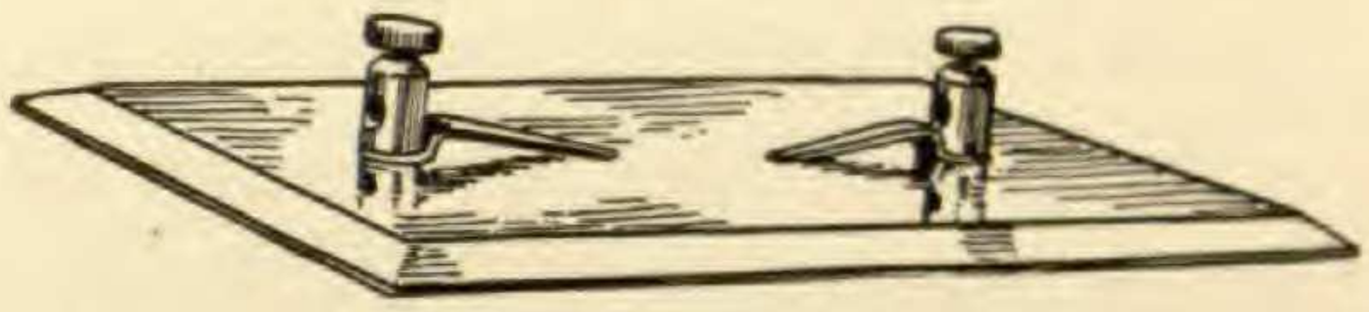

FIG. 5. Slide with binding posts.

Mercury reservoir.—Mercury is often serviceable and occasionally indispensable in physiological work. Sometimes, as in eudiometric experiments, it must be dry and perfectly clean. The several ways of cleaning mercury are mostly tedious and unsatisfactory, and repeated trials led finally to the adoption of a reservoir that keeps the mercury always dry, clean, and ready for use.

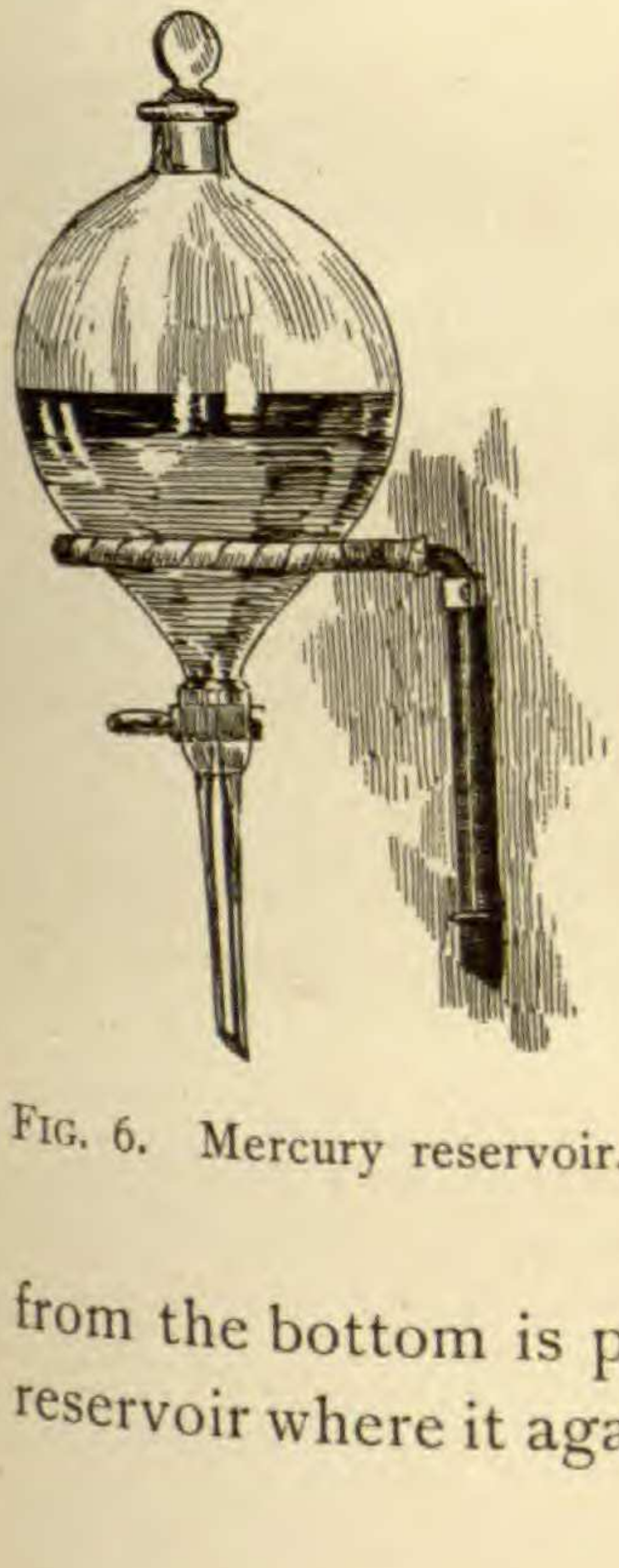

FIG. 6. Mercury reservoir.

The reservoir consists of a thick walled glass separatory funnel, about 15^{cm} in diameter (*fig. 6*). In this the mercury with some mercurous sulfate is placed, together with enough concentrated sulfuric acid to make a quarter-inch layer over the surface. To begin with it is shaken up several times, and in twenty-four hours is ready for use. Mercury drawn from the bottom is pure and dry. After use it is returned to the reservoir where it again becomes usable, without further attention.

The reservoir should be about half full in order to have as much surface of mercury exposed to the action of the acid as possible, and should be kept always stoppered. If mercurous sulfate is not at hand, it can be made by gently heating mercury with concentrated sulfuric acid (1^{cc} mercury to 10^{cc} acid), being careful that there is always an excess of acid. This should be done in a hood.

The reservoir may be placed against the wall by using a ring supported by staples, as shown in the cut.

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